# Natural Products Research in South Africa: End of an Era on Land or the Beginning of an Endless Opportunity in the Sea?†,‡

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ABSTRACT

**South African organic chemistry is deeply rooted in a rich history of natural product chemistry research with the secondary metabolites emanating from South Africa's unique floral kingdoms dominating this research for nearly a century. However, South Africa's terrestrial biodiversity is exceeded by an even greater diversity of marine flora and fauna occurring off its coast, which remain a relatively untapped source of novel biomolecules with unrealized pharmaceutical and agrochemical potential. Examples of the seemingly endless opportunities arising from exploration of the diverse bioactivities of South African plant and marine natural products, together with their semi-synthetic analogues, are presented here.**

KEYWORDS

**Marine natural products, diterpenes.**

## 1. Introduction

The first Frank Warren Lecture entitled 'Lingering amongst the Labiatae' was presented by the late Douglas Rivett at the inaugural Frank Warren Conference also held at the University of KwaZulu-Natal (formerly the University of Natal) in Pietermaritzburg in 1983.[1] One of the great characters or South African academia and a doyen of natural products chemistry in this country, Douglas Rivett, amongst many other achievements, was the first South African to publish research chemistry continuously over seven decades (1946–2005). Adapted from the 11th Frank Warren Lecture, this paper is dedicated to the memory of Douglas Rivett who sadly passed away shortly after the 2010 Frank Warren Lecture was delivered.

Rivett's inaugural Frank Warren lecture focused on the chemistry of labdane diterpene natural products from endemic South African plants of the 'mint' family (Lamiaceae – formerly Labiatae).[1] Since then natural products research in South Africa, in line with global trends, has been subjected to fluctuating fortunes largely driven by increasingly limited financial support for this field of research endeavour. With a history of over a century of active natural products research coupled to a vast and unique biodiversity, South Africa is well placed to exploit the recently predicted upswing in international natural products research.[2] This paper briefly overviews the history and present status of natural product research in South Africa and, taking examples from our two decades of research in the South African marine environment, will highlight the endless opportunities provided by a study of the marine natural products unique to this exceptionally biodiverse region of the world.

†Adapted from the 11th Frank Warren Lecture, Pietermaritzburg, 19 January 2010.

‡Dedicated to the memory of Douglas Eric Arthur Rivett (27 June 1921 – 25 January, 2010).

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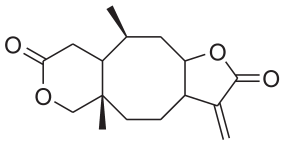
## 2. Plant Natural Products Research in South Africa – End of an Era?

Brown[3] and, more recently, Mulholland and Drewes[4] have drawn attention to the rich history of natural products research in South Africa and the pivotal role which studies of indigenous plant natural products have played in the development of South African organic chemistry. Emerging from the chemical literature, and the historical perspectives provided by Brown, Mullholland and Drewes, it is clear that there has not been a single era of natural product chemistry research in South Africa but rather three sequentially overlapping eras which will be referred to here as the poisonous plant era, the specialization era and the IKS era.

### 2.1. The Poisonous Plant Era (*ca.* 1902–1960)

The poisonous plant era was driven by the socio-economic imperatives of agriculture in early 20th century South Africa. Understandably, widespread livestock losses caused by the ingestion of the many, then largely unknown, poisonous plants that litter the South African veld were not sustainable in the developing, largely pastoral, South African agro-economy. For example an estimated one million sheep died from vomiting disease ('vermeersiekte') in the Karoo region of South Africa during the 1920s as a result of eating the plant *Geigeria aspera* the source of the emetic toxin, geigerin (**1**).[4] Instances of toxic plant poisoning were not limited to livestock and a case of bread poisoning in the Mossel Bay and George areas in 1933 was linked to contamination of bread flour with alkaloids from indigenous *Senecio* species.[3]

From as early as 1918 the role which *Senecio* plants also played in the onset of cirrhosis of the liver ('dunsiekte') in South African cattle and horses was well known.[3,4] While much of the early work on the chemistry of poisonous plants, e.g. *Senecio* species was carried out at Onderstepoort near Pretoria, research into plant toxins did not remain confined to this Government veteri-



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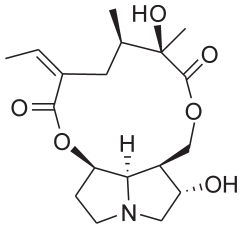

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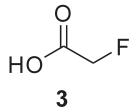


**3**

nary research facility as the 20th century unfolded. At the University of Natal, Frank Louis Warren, widely revered patriarch of South African organic chemistry, entered the endemic plant toxin field in 1943, with the publication of the chemical structure (*sans* relative or absolute configuration) of the pyrrolizidine alkaloid rosmarinine (**2**) isolated from *S. rosmarinifolius*.[5] The elucidation of the chemical structure of **2** ushered in for Warren a life's work in the pyrrolizidine alkaloid field that would bring him both national and international recognition.[3] Of all the toxins isolated from South African poisonous plants during this era, monofluroacetic acid (**3**) stands alone in its structural simplicity, potent toxicity and significance as a plant toxin lethal to all animals. Identified as the toxic principal in the endemic plant *Dichapetalum cymosum* ('gifblaar') **3** was the first fluorinated metabolite to be isolated from a plant and although it has now shown to be ubiquitous in many toxic tropical plants, interest in **3** and its source plant, *D. cymosum*, remains unabated after nearly a century of investigation.[3,4]

### 2.2. The Specialization Era (*ca.* 1960–1990)

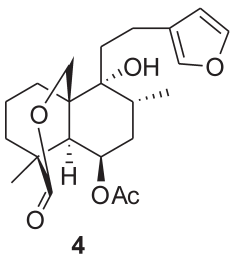
Fifteen years after the Second World War, in a time of relative prosperity coupled with increasing political turmoil in South Africa, an era dawned in South African natural product chemistry possibly best retrospectively described as the 'natural product specialization era.' Specialization took two forms. Firstly, specialization in terms of natural product investigations linked to a specific plant family, e.g. amongst others, Rivett[1,6] at Rhodes University (Lamiaceae) and Taylor[7] at the University of Natal in Durban (Meliaceae) and secondly, specialization within industrially and agriculturally important classes of natural products, e.g. flavonoids and proanthocyanidins (condensed tannins) at the University of the Orange Free State (Roux, Ferreira, Brandt, Bezuidenhout *et al.*)[8] and mycotoxins at the CSIR in Pretoria (Steyn, Vleggaar, van Heerden, Horak, Gorst-Allman *et al.*).[9] A plethora of new chemical entities emerged from this relatively well-resourced 'golden era' in South African natural product chemistry and a selection of the structurally diverse secondary metabolites isolated and identified by the individuals and groups *vide supra* include the *β*–furano-di and triterpenoids, dubiin (**4**)[6] and ekebergin (**5**)[7] from the Lamiaceae and Meliaceae, respectively, the proanthocyanidin robetinidol-(4*α*, 8)-catechin (**6**)[8] and the mycotoxin janthitrem (**7**).[9]

### 2.3. The IKS Era (*ca.* 1990–2020)

The advent of political and social change in South Africa in 1994 saw a shift in the focus of natural products research towards the study of traditional plant-based medicines as part of a wider indigenous knowledge systems (IKS) national research priority area. The natural products chemistry of traditional herbal remedies, however, was not a new area of research endeavour in South Africa and natural product studies of plants such as 'devil's claw' (*Harpagophytum* sp.) and the *Hoodia* cactus, sources of the anti-arthritic harpagoside (**8**)[10] and the triterpene glycoside appetite suppressant (**9**),[11] respectively, were initiated in the 1970s and were far advanced when this era dawned. The increasing worldwide interest, especially in Western Europe, in plant extracts as a form of alternative or complementary medicine, promises a bright future for South Africa's many traditional herbal medicines and this era, in all likelihood, is set to continue unabated over the next decade.

### 2.4. South African Natural Products in the International Drug Discovery Pipeline

Natural products still remain the mainstay of global new drug discovery, especially in the battle against cancer. A recent review of the contribution of natural products to anti-cancer drug



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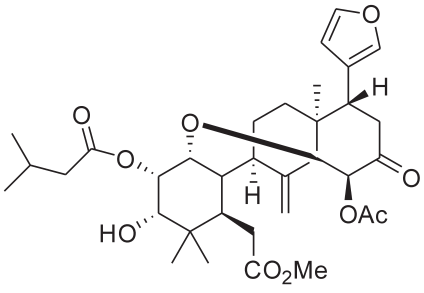

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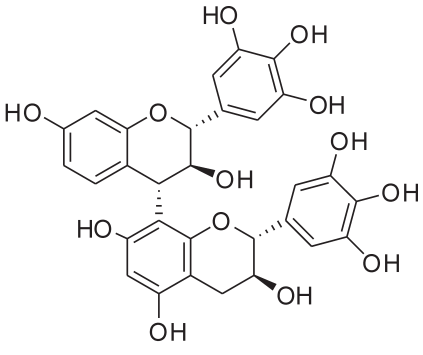

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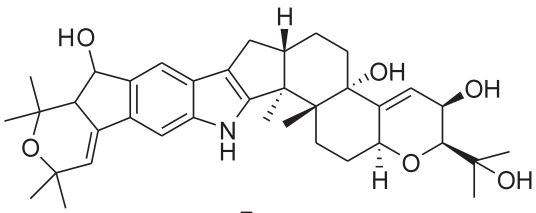

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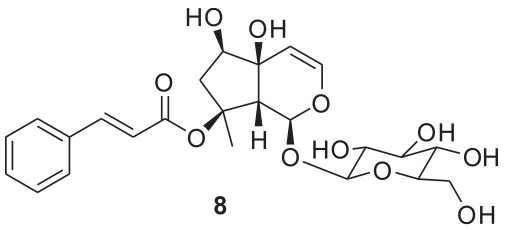

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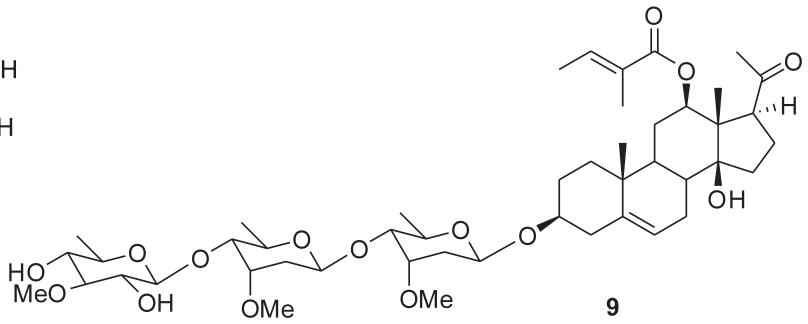

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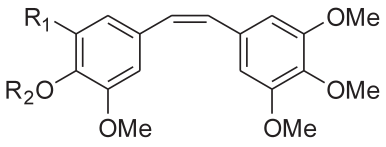
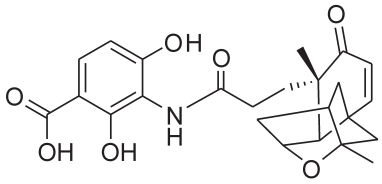


**10** $R_1 = H$, $R_2 = PO_3Na_2$
**12** $R_1 = OH$, $R_2 = H$



**11**

discovery reveals that 63 % of the anti-cancer drugs entering the market in the last 30 years have either a direct or indirect connection to natural products.[12] Paradoxically, despite southern Africa's position as the third most biodiverse region of the world,[13] only two South African natural products, fosbretabulin (combretastatin A4 phosphate, **10**) and platensimycin (**11**) are currently in the drug discovery pipeline. Combretastatin (**12**) was first isolated from the South African Cape bush willow (*Combretum caffrum*) by Pettit *et al.*[14] Currently under development as the anti-cancer drug (Zybrestat®) by the pharmaceutical company Oxigene, the phosphorylated analogue (**10**) of combretastatin is currently registered in a number of clinical trials including Phase III trials against thyroid cancer in combination chemotherapy with taxol and carboplatin.[15] Platensimycin (**11**) was discovered by Merck in 2006 from a soil isolate (*Streptomyces platensis*) collected in South Africa. Emerging from the systematic screening of approximately 250 000 natural product extracts (over 83 000 microbial strains cultured under three different growth conditions) **11** is one of the few compounds active against methicillin-resistant *Staphylococcus aureus* (MRSA) with no observed general toxicity and thus great promise as a future antibiotic.[16] The controversial *Hoodia* appetite suppressant **9**, licensed by the CSIR initially to Phytopharm and then to Pfizer, initially promised much but has yet to deliver on this promise.[10]

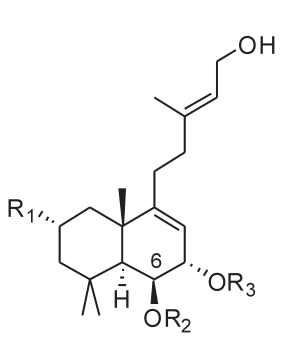
## 3. Marine Natural Products Research in South Africa – An Endless Opportunity?

The oceans, which cover 70 % of the earth's surface and comprise 95 % of its biosphere, remain the greatest unexploited cornucopia of biomolecular diversity on the planet and an ostensibly infinite source of new chemical entities with exploitable medicinal or agrochemical properties.[17] Many marine organisms (invertebrates, algae and microorganisms) produce natural products as a chemical defence against predation or in a chemically mediated response to, amongst others, interspecific competition for limited resources (e.g. space on a reef or access to nutrients) and intraspecific communication (e.g. larval settling cues). The coastline of southern Africa, stretching approximately 3000 km from southern Namibia in the west to southern Mozambique in the east, sustains a unique and relatively accessible diversity of endemic marine fauna and flora that can offer rich rewards for marine natural products chemists in search of novel bioactive secondary metabolites with possible medicinal properties. Over the last two decades the marine natural products research group at Rhodes University has isolated a plethora of new bioactive natural products from southern African marine organisms[18] and selected examples of the seemingly endless opportunities arising from our marine natural products research over this period are presented here.

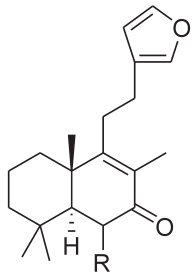
### 3.1. Limpets, Labdanes and Crop Diseases

The secretive mollusc, *Trimusculus costatus*, occurs in dense colonies hanging upside down under rock ledges in the intertidal zone near Cintsa on the southeast coast of South Africa.[19] By remaining hidden and possessing a shell, *T. costatus* is protected from terrestrial predators and desiccation during low tide. However, this mollusc also has to contend with the attentions of predatory fish, and carnivorous sea stars, when submerged at high tide for which the presence of a shell offers only limited protection. Interestingly, when subjected to predation, *Trimusculus* species, in common with some other shelled molluscs, are able to extend their inverted muscular 'foot' over the top of their shell to expose rows of small glands on the underside of the foot which produce a feeding-deterrent-containing white mucus to repel predators.[20]

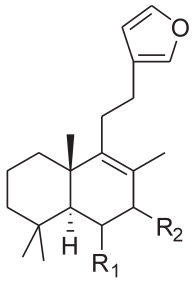
In a series of feeding assays using the common intertidal omnivorous predatory fish *Pomadasys commersonnii* we established that the two major feeding deterrents produced by *T. costatus* were the labdane diterpenes (**13** and **14**).[19,21] Of interest to us, given the well-documented history of labdane diterpene research at Rhodes University,[1] was the structural similarity between these two compounds and the labdane diterpenes hispanone (**15**) and rhinocerotinoic acid (**16**) isolated from two endemic South African plants of the Family Lamiaceae: *Ballota africana* and *Elytropappus rhinocerotis*, respectively.[22,23] Our attention was drawn to the sterically unfavourable, diaxial arrangement of the acetoxy functionalities at C-6 and C-7 in the



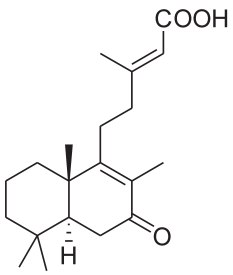

**13** $R_1$ = H, $R_2$ = $R_3$ = Ac
**14** $R_1$ = OAc, $R_2$ = $R_3$ = Ac




**15** R = H
**17** R = β–OH
**18** R = α–OH
**21** R = β-OAc




**19** $R_1$ = β-OH, $R_2$ = α-OH
**20** $R_1$ = α–OH, $R_2$ = β-OH
**22** $R_1$ = β–OAc, $R_2$ = β-OH

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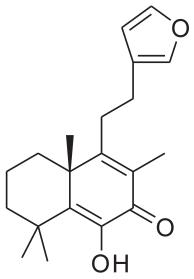


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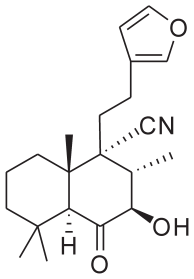


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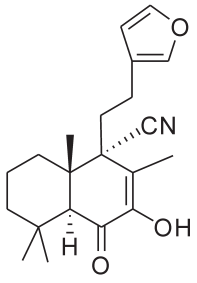



**25**

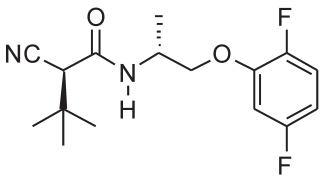
two *Trimusculus* metabolites, **13** and **14**, and we hypothesized that the introduction of a similar diaxial configuration of either acetoxy or hydroxy groups in **15** and **16** may enhance the bioactivity of these latter plant secondary metabolites.

From the outset we recognized the usefulness of the $\alpha,\beta$-unsaturated ketone moiety in directing further oxygenation at C-6 in **15** and **16**. Both C-6 hydroxy epimers (**17** and **18**) of the more readily available **15** were accordingly synthesized *via* Vedejs' oxidative procedure.[24] Standard hydride reduction of **17** yielded an epimeric mixture of **19** and **20** with the former more sterically hindered target compound, not unexpectedly, emerging in low yield (25 %).[25] Interestingly, sodium borohydride reduction of 6$\beta$-acetoxyhispanone (**21**), prepared *via* manganic acetate oxidation of **15**, afforded only 6$\beta$-acetoxy-7$\beta$-hydroxyhispanone (**22**).[25] Eager to increase the quantity of **19** available for bioactivity studies we proceeded to saponify **21** with ethanolic potassium hydroxide in an attempt to initially provide **17** which could then be reduced to **19** and **20**. To our surprise the major product of this saponification was the diosphenol (**23**) and not **17**, as anticipated.[25] Undeterred, we re-attempted the saponification of **21** using Mori's milder potassium cyanide saponification procedure to obtain, again unexpectedly, only the nitriles (**24** and **25**) as the major and minor products (45 and 9 %, respectively).[26] We subsequently proposed that **24** was formed through an initial stereospecific Michael addition of a cyanide nucleophile at C-9 in **21** followed by a 1,2-carbonyl transposition *via* a classical Lobry de Bruyn-van Ekenstein rearrangement of an $\alpha$-hydroxy ketone. Compounds **24** and **25** represented the first example of labdane diterpenes with a cyano substituent at C-9.

Opportunities to screen South African marine and plant natural products in a wide diversity of national and international biomedical and agrochemical screening programmes have been regularly presented to us over the last two decades. At the time we were involved in the *Trimusculus* research we were part of a collaborative agrochemical screening programme with Dow AgroSciences in the USA. Of the approximately 50 diterpene natural products and semi-synthetic derivatives (including **15**–**25**) submitted from our laboratory into this screening programme over a period of two years, only **24** and **25** exhibited significant activity in agrochemical screens against wheat rust (*Puccinia recondite*) and rice blast (*Magnaporthe grisea*) fungi.[26] The latter crop disease is responsible for the destruction of an estimated 50 % of the annual global rice crop and given the need for new chemical entities active against rice blast the activity of **24** observed in *in vitro* assays was deemed significant enough to warrant field trials against rice blast. While the initial *in planta* results were promising, **24** could not match the almost complete elimination of *M. grisea* infestation by the commercial fungicide azoxystrobin (**26**) at low concentrations (12.5 ppm).[26] Although the strategy to introduce vicinal diaxial oxygenation in ring B has not, as yet, afforded the increase in some form of bioactivity hoped for in **15** and **16** (we were unable to source this compound from local specimens of *E. rhinocerotis* and **16** was synthesized from commercially available sclareol),[27] the serendipitous discovery of the potentially useful fungicidal activity of **24** and **25** emphasizes the ongoing importance of attempting to add value to natural products, whether they are of plant or marine origin, through semisynthesis.

### 3.2. Sea Slugs, Malaria And Cancer

Sea slugs (also known as nudibranchs) are slow-moving, soft-bodied, shell-less marine molluscs which prey on other marine invertebrates, e.g. sponges and soft corals and do not possess any visible means of physical protection against predation, e.g. a shell or spines. However, their striking colouration warns potential predators that many of the sea slugs are chemically defended. While the majority of sea slug species sequester their chemical defence metabolites from the marine invertebrates on which they feed, a small number of these molluscs possess the ability to synthesize their defensive metabolites *de novo*, e.g.

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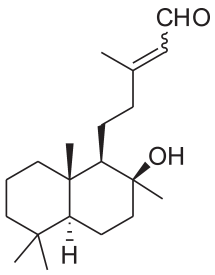
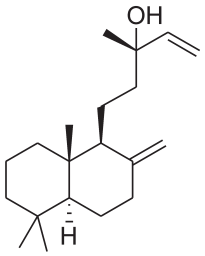



**27** $\Delta^{13}$ *E*
**28** $\Delta^{13}$ *Z*

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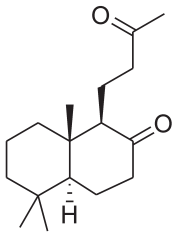



**30**

the Mediterranean sea slug, *Pleurobranchea meckelii*.[28]

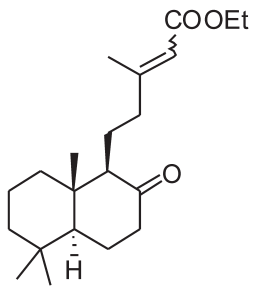
Given our interest in both terrestrial and marine labdane diterpenes our attention was drawn to the isolation of two reportedly unstable isomeric labdane diterpene aldehyde metabolites: labd-13*E*-ene-8$\beta$-ol-15-al (**27**) and labd-13*Z*-ene-8$\beta$-ol-15-al (**28**) from *P. meckelii* by Cimino and co-workers.[28] Bioactivity investigations of marine natural products, especially those isolated from nudibranchs, are frequently hampered by the paucities of these compounds that are often available for these studies.[29] Struggling with the facile isomerisation of **27** and **28** on exposure to standard chromatography media Cimino and co-workers were unable to isolate sufficient quantities of **27** and **28** for chemical ecology and bioactivity studies.[28] We therefore anticipated that the semisynthesis of these two compounds from a commercially available terrestrial plant diterpene, e.g. manool (**29**) could provide a possible solution to this particular marine diterpene supply problem.[30]

A key intermediate in our synthesis was the diketone (**30**), readily accessible from **29** *via* PCC oxidation followed by reductive ozonolysis. A Wittig-type reaction regiospecifically extended the side chain to give the esters (**31** and **32**) while a Grignard addition efficiently introduced the methyl group at C-8 with *Re*-facial selectivity. LAH reduction of the resultant hydroxy-esters (**33** and **34**) yielded the diols (**35** and **36**) which could be quantitatively converted into **27** and **28** by stirring with manganese dioxide. This final synthetic step provided a facile method to generate the natural products, **27** and **28**, from a stable precursor when required for chemical ecology or other bioactivity studies and thus avoided the isomerization issues associated with the chromatography of these compounds.[30]

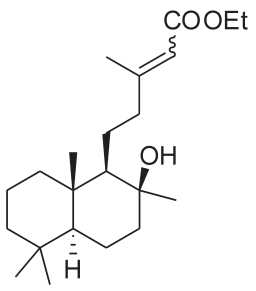
Again, the serendipitous discovery of a useful product from a side reaction during the synthesis of **31** and **32**, provided an unexpected opportunity to synthesize a new series of bioactive compounds.[31] We found that the addition of excess NaH during the Wittig step resulted in a quantitative conversion of **30** to the crystalline 8-hydroxy-13-podocarpanone (**37**) *via* an intramolecular aldol condensation reaction. 8-Hydroxy-13-podocarpanone provided us with a suitable precursor for the further Grignard and Wittig syntheses of a wide range of compounds (e.g. **38, 39** and **40**) analogous to the anti-plasmodial isopimarane diterpene (**41**) isolated from the bark of a Turkish tree.[32] Ongoing structure activity relationship studies have revealed that 8$\beta$, 13$\beta$-dihydroxy-13$\alpha$-phenylpodocarpane (**39**) yielded the most significant anti-plasmodial activity of the cohort of compounds prepared from **37** and was comparable with the activity reported for the naturally occurring substances. Interestingly, **39** also appeared to exhibit significant inhibition of *in vitro* $\beta$-hematin formation which may provide an initial clue as to a possible mechanism of action for these compounds against *Plasmodium falciparum.*

*Hypselodoris capensis* is a colourful sea slug inhabiting the sub-tidal reefs situated along the southern coast of South Africa. Our initial investigations of the sequestered chemistry of *H. capensis* revealed that this species obtained its major defensive chemicals ($\beta$-substituted furanosesterterpenes, e.g. variabillin, **42**) from a *Fasciospongia* sponge on which it was frequently observed feeding in the Tsitsikamma National Park.[33] Other metabolites isolated from this species of sea slug, e.g. the sesquiterpene nakafuran-8 (**43**), were not present in the *Fasciospongia* sponge and a return to our underwater study site in the Tsitsikamma National Park revealed that *H. capensis* occasionally also feeds on a *Dysidea* sponge which we were able to show was the source of **43** and another compound tsitsikammafuran (**44**).[34] Interestingly, **44** was not present in extracts of *H. capensis* suggesting a possibly selective uptake of sequestered metabolites by the sea slug. We were able to confirm the structure of **44** through the synthesis of both **44** and its regioisomer (**45**) through BuLi mediated halogen metal exchange (HME) reactions of the brominated precursors 3-bromo-*p*-cymene and 4-bromo-*m*-cymene with 3-furaldehyde.[34] The low yields (27–32 %) of the HME coupling reaction that afforded **44** and **45** are typical of HME reactions involving activated aromatic rings and we initially encountered similar low yields in our synthesis of analogues of the cytotoxic prenylated aromatic compounds isolated from the endemic South African sea slug *Leminda millecra.*

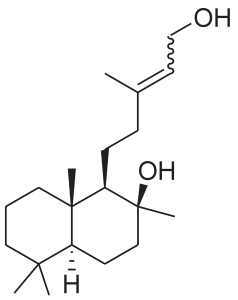
*Leminda millecra* is a common sea slug in Algoa Bay and sequesters a plethora of diverse bioactive metabolites from its diet of soft corals and sea fans.[35] Of interest to us was the cytotoxicity (8–60 $\mu$M) of several prenylated toluhydroquinones and toluqui-



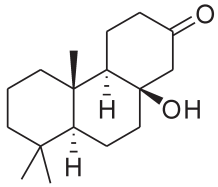

**31** $\Delta^{13}E$
**32** $\Delta^{13}Z$



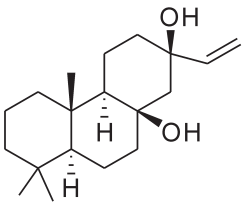

**33** $\Delta^{13}E$
**34** $\Delta^{13}Z$



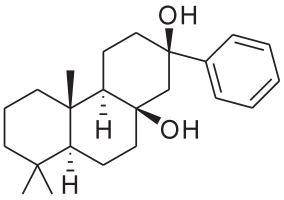

**35** $\Delta^{13}E$
**36** $\Delta^{13}Z$




**37**




**38**

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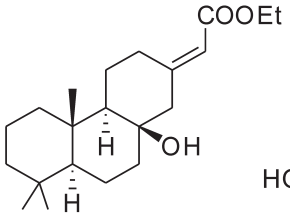
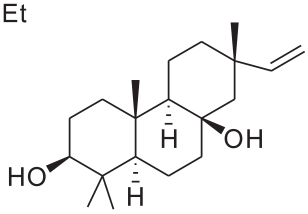



**40**




**41**

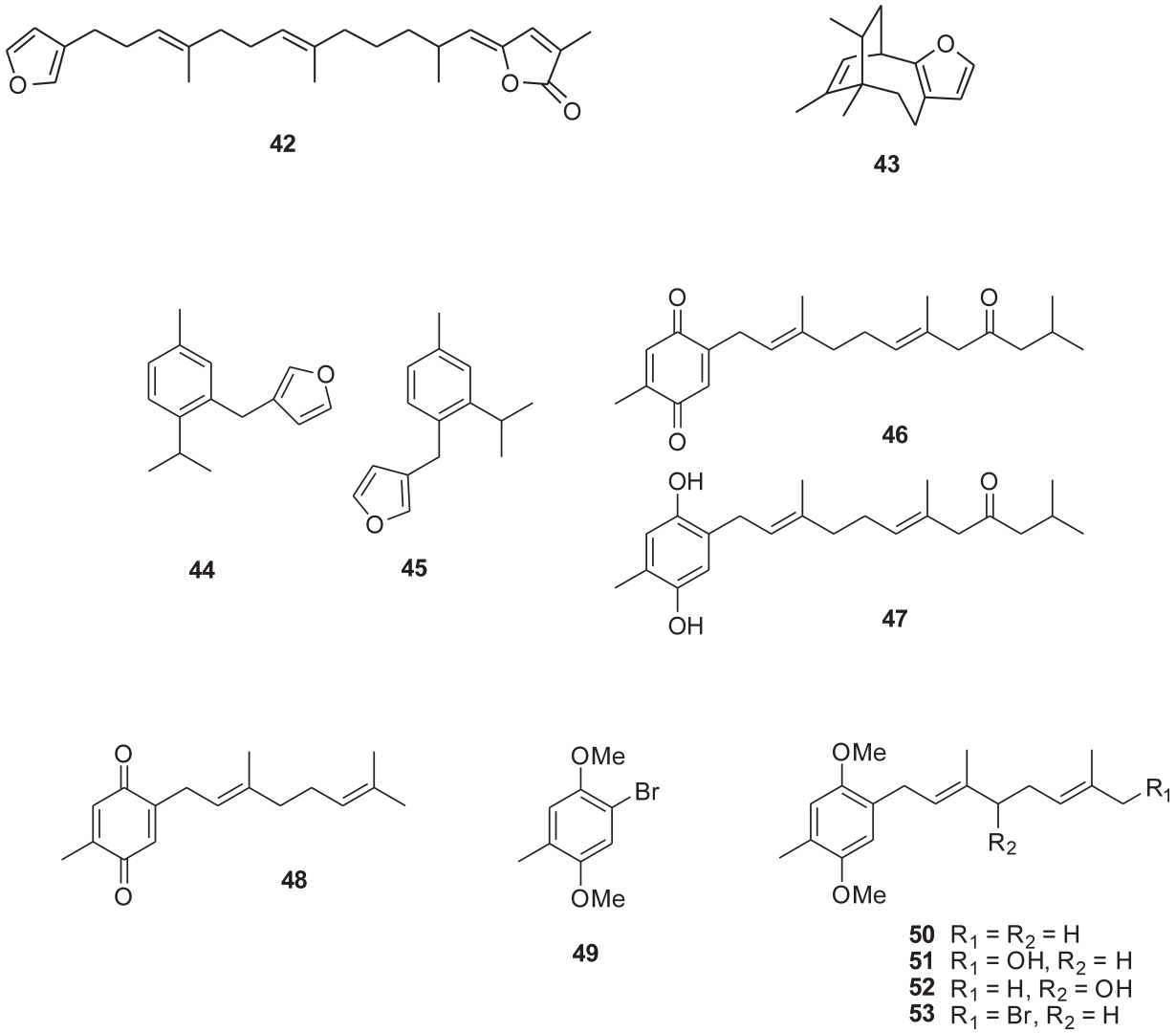
nones (e.g. **46** and **47**) to oesophageal cancer cells. Oesophageal cancer is particularly prevalent in the rural areas of the Eastern Cape and over the last six years we have routinely screened marine natural products against this form of cancer.[36] Using a cancer cell line (WHCO1), derived from a South African oesophageal cancer patient, we were able to establish the mechanism of action of **43** which induces caspase 3 mediated apoptosis of oesophageal cancer cells *via* the initial production of reactive oxygen species and subsequent activation of the c*Jun*/Erk signaling pathway.[37] The cytotoxicity observed for the prenylated quinones isolated from *L. millecra* prompted an SAR study of prenylated toluhydoquinones related to **46** and **47**. Synthesis of the simplified analogue (**48**), which proved to be similarly cytotoxic (6 $\mu$M) to **43** (8 $\mu$M), was achieved *via* an HME reaction between the aromatic bromide (**49**) and geranyl bromide followed by cerium ammonium nitrate mediated oxidative demethylation of the coupled product (**50**).[38] The capriciously variable and low yields of **50** (0–40 %) obtained from the HME reaction prompted us to investigate the mechanism of this reaction with a view to limiting the variability and improving the yield. A detailed $^{7}$Li and $^{13}$C NMR study of lithium aggregation occurring during this reaction enabled us to rationally change the reaction conditions to reproducibly afford **50** in 60–65 % yield.[39] This reasonably successful resolution of the challenges associated with the HME step provided an opportunity to synthesize the hydroxylated and brominated prenylated quinone precursors (e.g. **51**–**53**) which will be oxidatively methylated and screened against oesophageal cancer cells in due course.[38]

### 3.3. Squeezing New Opportunities Out of Marine Sponges

The secondary metabolites produced by marine sponges (Phylum Porifera) have provided the mainstay of global marine natural product chemistry for nearly half a century. South African sponges are no exception and they are an almost endless source of new bioactive compounds, e.g. the topoisomerase inhibitor, tsitsikammanine A (**54**)[40] and the autophagy modulating amino sterol (**55**).[41]

Sponges are filter feeders that feed on microbes and small particulate matter suspended in the water column. A sponge weighing only a kilogram is able to filter thousands of litres of sea water, rich in microbes, per day and it is therefore not surprising that large and diverse microbial communities are retained inside sponges which can, as reported for the giant barrel sponge *Xestospongia muta*, make up as much as 40 % of the internal volume of a sponge.[42] Over the last 15 years it has become increasingly apparent that many of the metabolites originally attributed to sponge biosynthesis are in fact the secondary metabolic products of the microbes that live in a symbiotic relationship with the sponge. In this relationship the sponge provides a living space and a source of nutrients for the microbes while the microbes supply the sponge with toxic secondary metabolites which are subsequently used as part of a chemical defence strategy against predation of joint benefit to both sponge and microbes. The factors that determine which microbes the sponge consumes and which are retained as symbionts are not clear.[42] From a natural product perspective a key indicator of a microbial, as opposed to a sponge, source for a marine natural product are the occurrence of either the same metabolite, e.g. manzamine A (**56**) in several unrelated species of sponge[43] or even in a different phylum, e.g. makaluvamine A (**57**) which was originally isolated from a latrunculid sponge (*Zyzzia* cf. *marsailis*)[44] and has subsequently also been reported from the fruiting bodies of a myxomycete (slime mould).[45]

Secondary metabolites containing a central pyrroloiminoquinone structural scaffold, e.g. **54** and **57** have dominated our studies of South African sponges from the Family Latrunculidae. South Africa and New Zealand are global centres of latrunculid sponge biodiversity and these green and brown pigmented sponges are common along the many reefs that line the South African coast. Pyrroloiminoquinone containing compounds are generally highly cytotoxic and discorhabdin A (**58**), isolated from a South African *Latrunculia* species, was found to be toxic to human colon tumour cells at very low concentrations (7 nM).[40] Regrettably, the apparent non-specific cytotoxicity of these compounds has hampered their development as anti-cancer drugs and we are unable to isolate sufficient quantities of these compounds to embark on semi-synthetic programmes in an



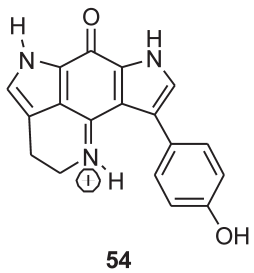

attempt to improve their selectivity and therapeutic index.

Given the well documented difficulties associated with extracting sufficient amounts of bioactive metabolites from marine organisms for clinical development, secondary metabolite producing marine microbes may hold the key to the long term development of bioactive marine natural products as new pharmaceuticals.[46] The isolation and culturing of marine microbes is not trivial and has its own associated difficulties.[42] However, our rapidly expanding knowledge of the gene clusters responsible for bioactive natural product biosynthesis provides an opportunity, once they have been identified, to transpose these clusters into a heterologous host more amenable to large scale propagation.[2] If this process is successful further opportunities arise to manipulate the relevant gene clusters in order to generate an additional library of 'unnatural' secondary metabolite analogues. As the unit cost of total genome sequencing continues to fall a significant shift in emphasis in global natural product research is predicted and the future rational targeting of those organisms with the genetic capacity to produce natural products of pharmaceutical interest may not be just a pipe-dream.[2]

Therefore, of current interest to us are the microbial communities which inhabit latrunculid sponges belonging to the endemic sponge genus *Tsitsikamma.* This endemic South African sponge genus, originally discovered in the Tsitsikamma National Park, has produced a number of bioactive pyrroloiminoquinone natural products including the topoisomerase inhibitor, **54**.[40] The close structural similarities between **54** and wakayin (**59**) a secondary metabolite found in a completely unrelated marine ascidian invertebrate (*Clavelina* sp.)[47] would possibly suggest a common microbial origin for these particular pyrroloiminoquinone metabolites. The first step in our quest to investigate a possible microbial source for **54** and related compounds has involved an investigation of the microbial community structure in different specimens of *T. favus* through analysis of microbial 16S RNA gene sequences isolated from the sponge tissue and water squeezed out of freshly collected sponges.[42] The second step will be to compare the microbial community structure of the two closely related *Tsitsikamma* species *T. pedunculata* and *T. scurra* looking for any overlap in resident microbial species which will hopefully provide insights into a possible common microbial source for the tsitsikammamines and other pyrroloiminoquinone metabolites.

## 4. Conclusion

Over the last decade the high-throughput screening of libraries of synthetic compounds in international pharma's drug discovery programmes has increasingly diverted attention away from natural products as a source of new drugs. Natural products are biosynthesized by proteins and are often required to bind to proteins to execute the bioactivities for which they are produced by a living organism. As proteins only have a limited number of fold types it should be explicit that many natural products have privileged structures to bind to protein receptors and therefore

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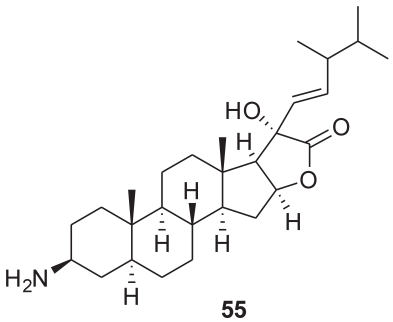


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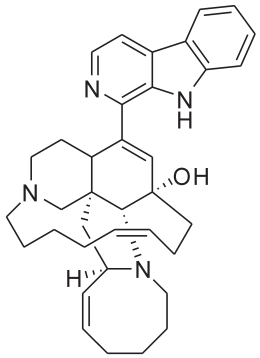
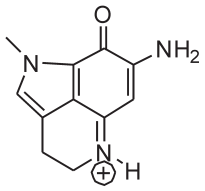



**56**

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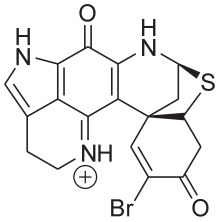
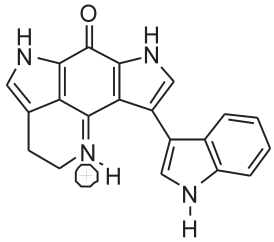



**58**




**59**

cannot be largely ignored as a source of new drugs for much longer.[2] Advances in separation and structure elucidation technologies coupled with a proliferation of new directed screening methods, rapid genetic sequencing and a growing realization of the potential of biosynthetic pathway exploitation to generate an almost limitless library of natural product analogues, is heralding a renaissance in international natural product based drug discovery.[2,12]

South Africa has a rich history of natural products chemistry research and is well placed to benefit from any renewed international interest in natural products as a source of new pharmaceuticals. However, a perception from the current allocation of research funding in South Africa would suggest that the future of natural products research will increasingly become the responsibility of botanists and ethnobotanists and this new development must be challenged by South Africa's organic chemists who have been the custodians of natural products chemistry in this country for over a century. Rigorous total structure elucidation of bioactive organic natural products is the basic prerequisite of internationally recognized natural product chemistry and is of crucial importance to both the pharmaceutical industry and synthetic chemists involved in natural product total synthesis. It is also the legacy we inherited from Warren, Rivett and others of their generations. Present and future South African organic chemists should neither ignore the endless opportunities associated with the unique biomolecular diversity inherent in southern Africa's enviable biological resources nor voluntarily abdicate their responsibility to lead natural products research in this country.

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